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Coccidiosis of Cattle

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Coccidiosis of Cattle

Some Unsolved Problems

The disease known as coccidiosis occurs in many domestic and wild animals. It is of great importance in chickens, in which coccidiosis is one of the chief causes of losses to the producer. In cattle the disease was estimated by Fitzgerald in 1962[1] to cause an annual loss of $3,500,000 in calves under one year of age in the 11 western states and $7,500,000 in the seven west north-central states. In making this estimate Fitzgerald calculated that 90 percent of all calves are infected by coccidia, and that the average loss amounted to 75 cents per head on all calves less than one year of age in the United States. Coccidiosis is observed throughout the year, but most commonly during the late fall and during winter and spring. It occurs more frequently in calves from about one to six months in age, but older animals, especially those one to two years old, are often affected. The signs include diarrhea, weakness, and lack of appetite. Blood may or may not be evident in the feces. In severe cases, the animals become emaciated, and death or retardation in growth results. Although bovine coccidiosis has been investigated for many years, numerous important problems in connection with this disease remain to be solved.

The agents which cause coccidiosis are protozoa (tiny one-celled animals), chiefly of the genus *Eimeria.* They belong to the same general group as the parasites causing malaria in man. The coccidia are unusual among parasites in their high degree of host-specificity, that is, the extent of limitation to a single host or few hosts. Some of the coccidia of cattle are found also in elk, zebu, and water buffalo, but the majority live only in cattle; certain of the species in sheep occur in goats and certain wild rumi-

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these parasites. Therefore, it is likely that these 539 described species of *Eimeria* represent only about 1.5 percent of the total number present in chordates, according to Levine (1962). In hosts such as the rabbit, in which careful studies have been made, each species was found to differ in life cycle and in exact location in the host from all of the other species parasitizing that host (Cheissin, 1957).

Most of the species of bovine coccidia present in the United States were first carefully described by Christensen (1941). Nine species are known to occur in Utah, including *E. bovis, E. zurnii, E. ellipsoidalis, E. auburnensis, E. cylindrica, E. subspherica, E. canadensis, E. bukidnonensis, and E. alabamensis.* The four first-named species occur most frequently. Only two of these species, *E. bovis* and *E. zurnii,* are known regularly to cause coccidiosis accompanied by bloody diarrhea. Low-level infection with one or several species of coccidia is normally present in cattle, with no apparent damage to the host. Examinations for coccidia are made by collecting fecal samples, and mixing with concentrated sugar solution to cause the coccidia to float to the surface. The mixed samples are examined in special glass slides under the microscope. In this examination an estimate as to the number of each coccidial species present must be made to ascertain the possible importance to the health of the host animal.

LIFE CYCLES

Coccidia have a complex life cycle, with several generations included in a single cycle. The stage found in the feces is the oocyst, which has a protective wall, resistant to physical, chemical, and bacterial action. Oocysts freshly discharged in the feces must undergo a developmental process, called sporulation, before they become infective to another animal. This process, occurring outside of the host, requires at least 2 to 3 days and results in the for-

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schizonts, the first parasitizing the endothelial cells of the lacteals centrally located in the villi of the small intestine, and the second, the epithelial cells lining the crypts of the large intestine. The first-generation schizont is so large (mean greatest diameter, nearly 0.3 mm) that it can be seen without the aid of a microscope, requires about 2 weeks to complete development, and each schizont has more than 100,000 merozoites (Hammond et al., 1946). The second-generation schizont is much smaller (about 0.01 mm in greatest diameter), contains 30 to 36 merozoites, and requires only 1½ to 2 days to develop (Hammond, Andersen, and Miner, 1963). Thus, each infective oocyst ingested by a calf has the potentiality of yielding approximately 24 million second-generation merozoites (8 x 100,000 x 30). If this potentiality were fully realized, ingestion of only 1,000 such oocysts could result in the destruction of 24 billion intestinal cells.

The second-generation merozoites enter new host cells, and undergo sexual reproduction, culminating in the formation of oocysts. The oocyst is formed by union of 2 sexual cells, one egg-like (macrogamete) and one sperm-like (microgamete). The macrogamete arises by a growth process from a precursor called the macrogametocyte, whereas the microgametes arise by a reproductive process from a precursor called the microgametocyte, each of which produces many microgametes. The microgametes are actively motile; they move by means of 2 long flagella attached to the anterior end of a slender body. This final, sexual generation matures in about 3 days, so that all of the life cycle stages within the host are completed in a minimum of about 18 days, with the peak in numbers of oocysts discharged coming 19 to 22 days after ingestion of oocysts. The individuals of the sexual generation are more numerous than those of the previous stages, and are more injurious to their host cells, causing bleeding and partial destruction of the mucous lining of the large intestine. The signs of coc-

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oocysts apparently ready to be discharged were found 19 days after inoculation. All of the stages found were in epithelial cells. The pathological changes noted by Davis and Bowman included a sloughing of the epithelium lining the intestine and regional destruction of crypts, followed by necrosis. E. *zurnii* seems to cause coccidiosis more frequently in older animals than does *E. bovis*. This species is the one chiefly involved in the manifestation of the disease known as "winter coccidiosis," which usually occurs during or following cold or stormy weather in the winter months.

In *E. auburnensis* there is a large schizont resembling in general that of *E. bovis* in size and location, except for being more deeply imbedded (Davis and Bowman, 1962). The number of asexual generations has not been determined. The sexual stages are unusual in that they parasitize cells of mesodermal origin, lying beneath the epithelium of the villi in the small intestine (Hammond, Clark, and Miner, 1961). The microgametocytes are unusually large, reaching a size such that they can be seen without the aid of a microscope, and each produces thousands of microgametes. The life cycle stages within the host are completed in about 18 days. The oocysts, which are developed in the interior of villi, cannot reach the lumen of the intestine and be discharged from the host without the breaking or sloughing off of the epithelial layer.

E. ellipsoidalis also develops in the small intestine, but all of the known stages occur in the epithelial cells lining the crypts (Hammond, Sayin, and Miner, 1963). These schizonts are relatively small, containing only 24 to 36 merozoites; the number of generations is unknown. The internal portion of the life cycle is relatively short, inasmuch as only about 10 days are required for its completion. In severe infections a non-bloody diarrhea, usually lasting only a few days, may occur.

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the lower small intestine in a calf killed 25 days after inoculation. Nothing is known of the internal stages of the life history of the other bovine species. There is no evidence that any of these are pathogenic.

The species of coccidia are differentiated primarily by morphology of the oocysts, which differ in size, shape, color, and other features. The sporulated oocyst is especially useful, because it has more characters than the unsporulated oocyst. Levine (1963) has calculated that 2,654,736 morphologically different oocysts are possible for *Eimeria*. If available, information concerning the stages living within the host, host-specificity, and immunity may also be used to distinguish species.

Coccidiosis differs from bacterial diseases and from such protozoan diseases as malaria in that the severity of infection is more dependent upon the number of organisms which initiate the infection. The potential for multiplication of the coccidia is self-limited; thus the infection stops spontaneously after the life cycle is completed.

The prevalence of natural infections with coccidia in cattle makes it difficult to study coccidial infections in these animals experimentally. It is not feasible to rear cattle in such a way that natural infections are eliminated completely, but the occurrence of these in young calves can be greatly reduced by careful sanitation. In chickens, rabbits, and turkeys, which can be grown experimentally without natural coccidial infections, it has been possible to infect animals with single oocysts and determine the number of oocysts discharged from the host as a result of this infection. Also strains of these coccidia originating from a single oocyst can be obtained in this way for experimental use. This has not yet been done with coccidia of cattle.

If single oocyst infections could be caused in calves it would be possible to obtain more precise information about the life cy-

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In each infective, sporulated oocyst of the genus *Eimeria,* the eight sporozoites are arranged in groups of two. Each pair of sporozoites, with its surrounding delicate membrane, is called a sporocyst. The four sporocysts are enclosed within the double-layered oocysts wall, which is relatively thick and tough. In beginning a new infection, the sporozoites become active and escape from their confinement within the sporocyst and oocyst by a process called excystation. This ordinarily is completed in the intestine of the host.

Excystation has long been a subject of interest and study. Smetana (1933) found that oocysts of *E. stiedae,* which infect the liver of rabbits, could be made to excyst outside the host by using pancreatic juices, with the active agent being trypsin. In working with the same species, Goodrich (1944) could not obtain excystation unless the oocysts were first broken by mechanical means, freeing the sporozoites.

Working with sheep coccidia, Lotze and Leek discovered (1960) that bile aided excystation. Jackson (1962) found that intact oocysts would readily excyst if treated with carbon dioxide under anaerobic conditions in the presence of a reducing agent, before incubation in bile-trypsin mixtures. After pretreatment with carbon dioxide, the oocysts were found to have undergone a morphological change in an area at one end of the oocyst, known as the micropyle, where the wall is relatively thin. The permeability of the wall in such oocysts was markedly increased. In our laboratory, similar results were obtained with several species of bovine coccidia (Nyberg and Hammond, 1964). It is likely that the carbon dioxide acts as a stimulus resulting in production of an enzyme, which causes a change in the appearance and permeability of the micropyle. Inside the host, this process probably takes place in the rumen, where high carbon dioxide levels and other

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which they regularly encounter in their hosts. When oocysts are ingested they are first usually taken into the rumen, although if ingested with liquids they may bypass the rumen and enter directly into the abomasum, which corresponds with the stomach of other animals. In the rumen the oocysts are exposed to carbon dioxide, to which they respond by alteration of the micropyle. While in the rumen, the oocysts may also be subjected to regurgitation and mastication, which would probably result in some breakage of the oocyst walls. Such a mechanical breakage would be similar to that thought by Doran and Farr (1962) to be an essential part of the excystation process in species of coccidia living in gallinaceous birds with a gizzard. This is probably a much less important factor in coccidia living in ruminants. We have found that infection with *E. bovis* occurs equally well if oocysts are introduced into the abomasum, rumen, or mouth (Hammond, McCowin, and Shupe, 1954). Thus, neither the mechanical action of mastication nor the exposure to carbon dioxide which is associated with passage through the rumen appears to be essential to excystation of this species. In oocysts bypassing the rumen, exposure to carbon dioxide may occur in the abomasum or intestine, or some other stimulus may bring about alteration of the micropyle.

When oocysts with altered micropyles reach the small intestine, they respond to action of bile and trypsin by disappearance of the Stieda bodies, which resemble plugs at the tip of the sporocysts. At this time the sporozoites begin moving inside the sporocysts, and soon the two sporozoites in turn squeeze through the relatively small opening left by the disappearance of the Stieda body. After moving around inside the oocyst the sporozoites push their way through the micropyle. They then are free to penetrate the epithelium lining the intestinal wall and begin development into schizonts.

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without any trypsin. Boiled bile did not induce excystation; autoclaved sodium taurocholate caused movement of the sporozoites, but no complete excystation. Further work must be done before any conclusions can be drawn.

The differences in the excystation process in the various species of bovine coccidia, and in coccidia of other hosts are not entirely known. In the work we have done thus far no striking differences have been found in *E. bovis*, *E. auburnensis*, and *E. ellipsoidalis*. Possibly *E. zurnii* will be found to show some differences, because of the different patterns of response of the host to inoculation with this species.

Coccidia of other hosts may be found to exhibit differences in the excystation process. However, Lotze and Leek (1963) found that species from sheep would excyst when inoculated into rabbits, white rats, hamsters, and a turkey. The chief difference they observed among coccidia of different hosts was that many oocysts of *E. tenella* of chickens were ruptured in passing through the gizzard of turkeys and chickens, but few if any of the oocysts of sheep coccidia were so ruptured. They suggested that the process of excystation was sufficiently similar in coccidia from different hosts, so that this could not explain limitation of the parasites to a single host. However, this subject requires further investigation, because Koyama (1959) has reported finding marked differences in the responses of coccidia from rabbits, chickens, and sheep to exposure to pancreatic enzymes, with or without mechanical breakage of the oocysts. Also Marquardt (1963) found that the sporozoites of *E. nieschulzi* of the rat became active when the oocysts were crushed, without any other stimulus being necessary.

INVASION OF HOST CELLS

After the sporozoites have completed the process of excystation, they invade the intestinal wall. Nothing is known about the process of invasion of a host cell by sporozoites or merozoites of

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is *E. alabamensis*. In this species, Davis et al. (1957) found sporozoites in the distal end of cells in the tips of villi in the small intestine of a calf killed 2 days after inoculation. The route of invasion of sporozoites of *E. bovis* is now known. We found early schizonts in calves killed 5 or 6 days after inoculation of oocysts, but none in a number of calves killed 2, 3, or 4 days after inoculation (Hammond et al., 1946). It is difficult to explain the delayed appearance of the schizonts in this species. Excystation is not unduly delayed, because sporozoites were found in the contents of the small intestine in calves killed 15 to 18 hours after inoculation. The finding that macrophages are involved in the invasion route of certain species of chicken coccidia (VanDoorninck and Becker, 1957; Patillo, 1959; Challey and Burns, 1959) suggests a possible factor to explain the delay in the appearance of the schizonts. If the sporozoites are engulfed by macrophages and transported to the appropriate host cells (endothelial cells lining the lacteals of the villi) this may require more time than would a more direct route.

EFFECT ON HOST

Little progress has been made in describing or understanding the pathological changes caused by coccidiosis in cattle since the pioneering work of Smith and Graybill (1918). A detailed description of these changes is needed, as well as information about the influence of weather, nutrition, and bacterial flora on the severity of infection. Clark and Smith (1963) have shown that the course of infection with *E. tenella* in germ-free chickens is essentially the same as in conventional chickens. Recently, Davis, Herlich, and Bowman (1959a, 1959b, 1960a, and 1960b) have found that concurrent infections with certain intestinal worms enhance the development of coccidia in cattle and increase the

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As the first-generation schizont of *E. bovis* grows inside its host cell, this cell shows alterations from its normal appearance. Frequently, the nucleus becomes enlarged and displaced to one side of the cell by the growing parasite, and the chromatin often assumes a more coarsely granular appearance than normal. As suggested by Levine (1963), the nature of such enlargement of the host cell nucleus is unknown. The investigation of this might provide a useful approach in studying nuclear development and cytogenetics. In the late stages of growth, the cytoplasm of the host cell is stretched out into a thin, shell-like layer covering the schizont. The nucleus, resembling the setting of a ring, is flattened and its chromatin shows degeneration, being arranged in large clumps. The parasitized villus becomes swollen, and presumably the covering epithelium breaks or sloughs off at the time the merozoites are discharged from the schizont. The small, second-generation schizont causes relatively little alteration of its host cell during the early and intermediate stages of growth. However, these host cells are presumably destroyed by the rupture of the mature schizont and the discharge of its merozoites into the lumen of the crypt. In contrast, the gametocytes cause a marked alteration of their host cells, even in the relatively early stages of growth. A shrinkage and change in shape occur, so that an infected cell loses contact with its neighbors except at its base, thus disturbing the typical columnar arrangement. The gametocyte stage of *E. bovis* therefore appears to be more pathogenic individually than the other stages of the life cycle. Inasmuch as the gametocytes are also more numerous than any of the other stages, it is easy to understand why damage to the host is associated primarily with this stage.

In severe infections the majority of the crypts are destroyed, the epithelial layer is denuded, and much blood is lost. The course of the disease indicates that toxins are released by the coccidia, clumps. The parasitized villus becomes swollen, and presumably the covering epithelium breaks or sloughs off at the time the merozoites are discharged from the schizont. The small, second-generation schizont causes relatively little alteration of its host cell during the early and intermediate stages of growth. However, these host cells are presumably destroyed by the rupture of the mature schizont and the discharge of its merozoites into the lumen of the crypt. In contrast, the gametocytes cause a marked alteration of their host cells, even in the relatively early stages of growth. A shrinkage and change in shape occur, so that an infected cell loses contact with its neighbors except at its base, thus disturbing the typical columnar arrangement. The gametocyte stage of *E. bovis* therefore appears to be more pathogenic individually than the other stages of the life cycle. Inasmuch as the gametocytes are also more numerous than any of the other stages, it is easy to understand why damage to the host is associated pri-

distribution, then degenerative changes characteristic of those which result from toxins or lack of oxygen. In the later stages of growth of the parasites, the cytoplasm of the host cell appeared to undergo dissolution, a change which facilitates movement inside the host cells of the microgametes, preparatory to fertilization.

A comparative study of the injury caused by the several different species of bovine coccidia should yield information of fundamental value in understanding the host-parasite relationship.

CYTOLOGY

The coccidia provide interesting material for cytological study. Recently, this study has been facilitated by use of the newer techniques, such as phase contrast, fluorescence, electron microscopy, and cytochemistry.

Oocysts are difficult to study cytologically because their shells are impermeable to most of the agents used for fixing and staining. Davis and Bowman (1963) have removed the oocyst walls with antiformin, allowing greater penetration of acridine orange and other fluorescent stains. Another approach to this problem is to section the oocysts, and we are currently obtaining promising results with this method.

Sporozoites may be obtained for study by inducing oocysts to excyst *in vitro*. After leaving the oocyst, the sporozoites are actively motile for some time, intermittently undergoing the gliding movement characteristic of sporozoa, and a flexing movement of the anterior end. The sporozoites of *E. auburnensis* and *E. bovis* have a rather small nucleus, located near the center of the slender body, and anterior and posterior to this are two large spherical or ellipsoidal, refractile bodies. In *E. auburnensis* sometimes a third, distinctively smaller, refractile body is visible. In

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ment proceeds. The significance of this is not known. The nucleus has an eccentric endosome. As the schizont grows, nuclear division occurs repeatedly, resulting in the formation of many daughter nuclei. Finally, merozoites are formed.

The merozoites resulting from the giant schizonts of *E. bovis* are obtainable in large numbers for study. Living merozoites move actively, in a manner resembling that of the sporozoites. Morphological details are difficult to observe in living merozoites, even with the phase-contrast microscope. By using a protargol staining method a pore can be seen at the anterior end, and a median tubule can sometimes be demonstrated leading posteriorly from this pore. A similar pore was found in *E. meleagrimitis* merozoites by Augustin and Ridges (1963). Internal tube or rod-like structures have been demonstrated by electron microscopy in the merozoites of *E. intestinalis* by Mossevitch and Cheissin (1961) and in the sporozoites of *Plasmodium, Lankesterella,* and other sporozoa by Garnham, Baker, and Bird (1962, 1963). The former authors ascribed a supporting function to these structures, whereas the latter authors suggested a glandular function associated with the penetration of host cells.

In merozoites stained with acridine orange and observed with a fluorescence microscope, the posteriorly located nucleus appeared green, indicating presence of DNA. Prominent red granules, indicating presence of RNA, were seen in the middle region of the merozoite, with one at the posterior extremity. There was also a suggestion of one or more RNA-containing granules centrally located in the nucleus, but this could not be determined with certainty because of the masking by the brilliant green fluorescence. Davis and Bowman (1963) reported that the polysaccharide content of merozoites increased with age.

The early macrogametocyte of *E. auburnensis* has a nucleus with a prominent spherical eccentric endosome, and nucleoplasm

merozoites by Augustin and Ridges (1963). Internal tube or rod-like structures have been demonstrated by electron microscopy in the merozoites of *E. intestinalis* by Mossevitch and Cheissin (1961) and in the sporozoites of *Plasmodium, Lankesterella,* and other sporozoa by Garnham, Baker, and Bird (1962, 1963). The former authors ascribed a supporting function to these structures, whereas the latter authors suggested a glandular function associated with the penetration of host cells.

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granules appear in the cytoplasm. These later help in the formation of the oocyst wall. Scholtyseck and Shaefer (1963) have found that the surface of growing macrogametocytes of *E. perforans* of the rabbit is covered with numerous fine tube-like protrusions, visible only with the electron microscope. These were thought to have the function of increasing the absorptive surface of the parasite.

Microgametocytes can be distinguished in an early stage in *E. auburnensis* by the presence of more than one nucleus. The process of nuclear division has not yet been studied. As nuclei become more numerous, they typically become arranged at the surface of the cell, but in the large microgametocyte of *E. auburnensis* numerous bodies, usually more or less spherical, are formed within the microgametocyte, and each has nuclei at its surface. The center of these bodies later undergoes dissolution, and each nucleus transforms into a microgamete. The microgametes may become motile while still in the microgametocyte. After becoming free, the microgametes must make their way through the tissue of the host (lamina propria of the villus) to a host cell containing a macrogamete, and enter this cell to effect fertilization. In other species, such as *E. bovis*, the microgametes are liberated into the lumen of a crypt and host cells infected with macrogametes are usually in the immediate vicinity. The greater number of microgametes produced by *E. auburnensis* is probably an adaptation to the more serious obstacles to be overcome by the microgametes of this species. Recently, Cheissin (1964) has studied the microgametes of *E. intestinalis* of the rabbit with the electron microscope. He found that the nucleus occupied about the posterior two-thirds of the body. In the anterior third of the body, a large mitochondrion was located, and the two posteriorly directed flagella originated from the anterior extremity. Scholtyseck and Spiecker (1964) observed a similar makeup in the microgametes

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At first the protoplasm of the zygote fills the entire space within the oocyst walls, but soon the protoplasm contracts into a more or less spherical body, called the sporont. The remaining space within the oocyst wall is presumably occupied by a nearly colorless fluid. In each of the bovine species, oocysts are occasionally seen in which the contraction of the sporont has not occurred. These abnormal oocysts evidently do not undergo any further development. It might be supposed that they represent specimens in which fertilization did not occur. However, fertilization is thought to be a stimulus necessary for formation of the oocyst wall. If this is correct, the oocyst stage would not be attained unless fertilization occurred. The process of fertilization has not yet been observed in any of the bovine coccidia. Further work must be done before these abnormal oocysts can be explained.

Little is known of the chromosome cycle of any of the coccidia belonging to the genus *Eimeria* or any closely related form, except for the recent report of Scholtyseck (1963b). In this study of *E. maxima* of the chicken, five chromosomes were seen in a body originating from the microgamete in an early zygote. In accordance with the cycle known to occur in related organisms, *E. maxima* is therefore thought to have a haploid number of five chromosomes. Only the zygote is considered to be diploid; reduction of chromosomes to the haploid condition probably occurs during the first division after fertilization. Chromosomes are difficult to demonstrate in the coccidia, but more information about these in the various stages of the life cycle is badly needed.

A problem associated with the chromosome cycle is that of sexual differentation. Nothing is known as to when and how the development of merozoites or early gametocytes is influenced in such a way that they become either microgametocytes or macrogametocytes. This is still a wide open problem, but Canning (1962) working with *Barrouxia schneideri*, which infects centi-

been observed in any of the bovine coccidia. Further work must be done before these abnormal oocysts can be explained.

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A problem associated with the chromosome cycle is that of sexual differentation. Nothing is known as to when and how the

microgametocyte and a macrogametocyte have been observed, indicating the likelihood that influences from the host cell do not play a part in sexual differentation. The problems of the mechanism of sexual differentation and of the development into the successive stages of the life cycle remain as fascinating challenges for future work.

IMMUNOLOGY

Animals infected with *E. bovis* develop resistance to reinfection (Senger et al., 1959). Precise information as to the duration of immunity is not available, but it remains at least three to six months, and may last a year or longer. Little is known about immunity to other species of bovine coccidia, but Davis et al. (1955) found that infections with *E. alabamensis* caused little immunity. Wilson and Morley (1933) reported a resistance to reinfection with *E. zurnii* in two calves. Evidence of immunity in calves experimentally infected with *E. ellipsoidalis* has been reported (Hammond, Sayin, and Miner, 1963).

In investigating the nature of immunity to bovine coccidia, we have attempted to learn which stages of the life cycle are affected by the immune reaction. The results of this work (Hammond, Andersen, Miner, 1963) indicated that the first-generation schizonts and/or merozoites, occurring in the small intestine, as well as the second-generation schizonts, merozoites, and gametocytes occurring in the large intestine, are affected by the immune reaction. However, the effect on the stages in the large intestine was found to be of greater importance than that on the stages in the small intestine. It was also found that the immune reaction affects the numbers but not the timing of the various life cycle stages. Some oocysts of *E. bovis* were found to be retained in the mucosa for several weeks after the time they are normally dis-

immunity to other species of bovine coccidia, but Davis et al. (1955) found that infections with *E. alabamensis* caused little immunity. Wilson and Morley (1933) reported a resistance to reinfection with *E. zurnii* in two calves. Evidence of immunity in calves experimentally infected with *E. ellipsoidalis* has been reported (Hammond, Sayin, and Miner, 1963).

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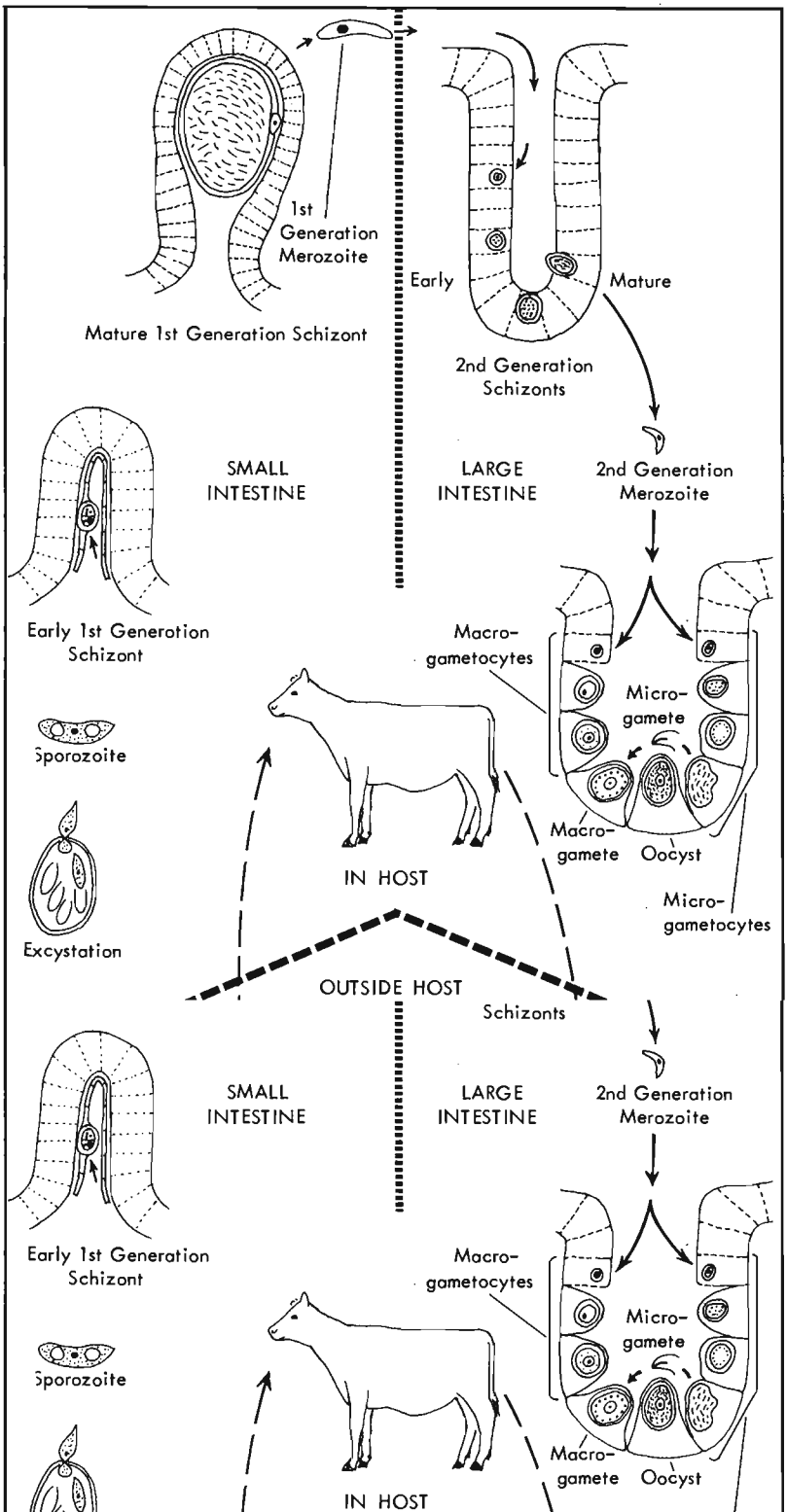
1st
Generation
Merozoite
Mature 1st Generation Schizont
Early
Mature
2nd Generation
Schizonts
SMALL
INTESTINE
LARGE
INTESTINE
2nd Generation
Merozoite
Early 1st Generation
Schizont
Macro-
gametocytes
Micro-
gamete
Sporozoite
Macro-
gamete
Oocyst
IN HOST
Micro-
gametocytes
Excystation
OUTSIDE HOST
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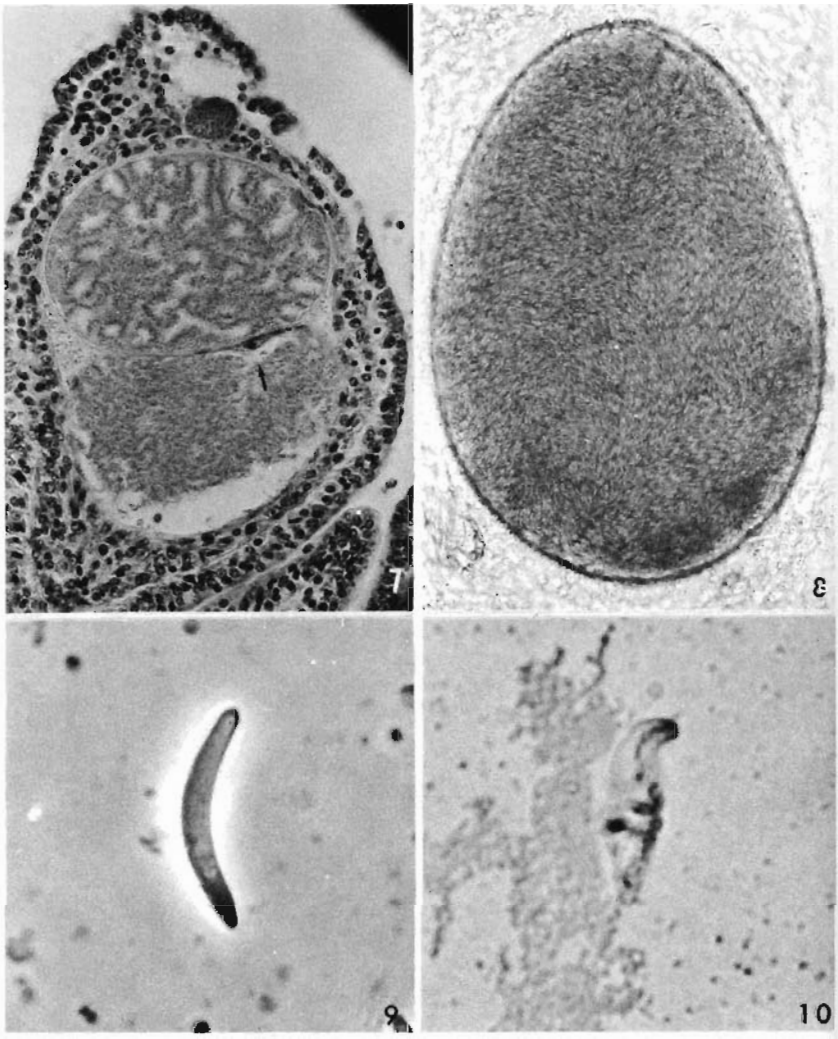

Fig. 7. Section of villus, with two nearly mature first-generation schizonts, one showing the host cell nucleus (arrow); fixed in Zenker's and stained with iron-hematoxylin, X150. Fig. 8. Mature first-generation schizont; fresh specimen, X200. Fig. 9. First-generation merozoite; fresh specimen, phase contrast, X3000. Fig. 10. Protargol preparation of first-generation merozoite, with tube- or rod-like structure in anterior end; X3000.

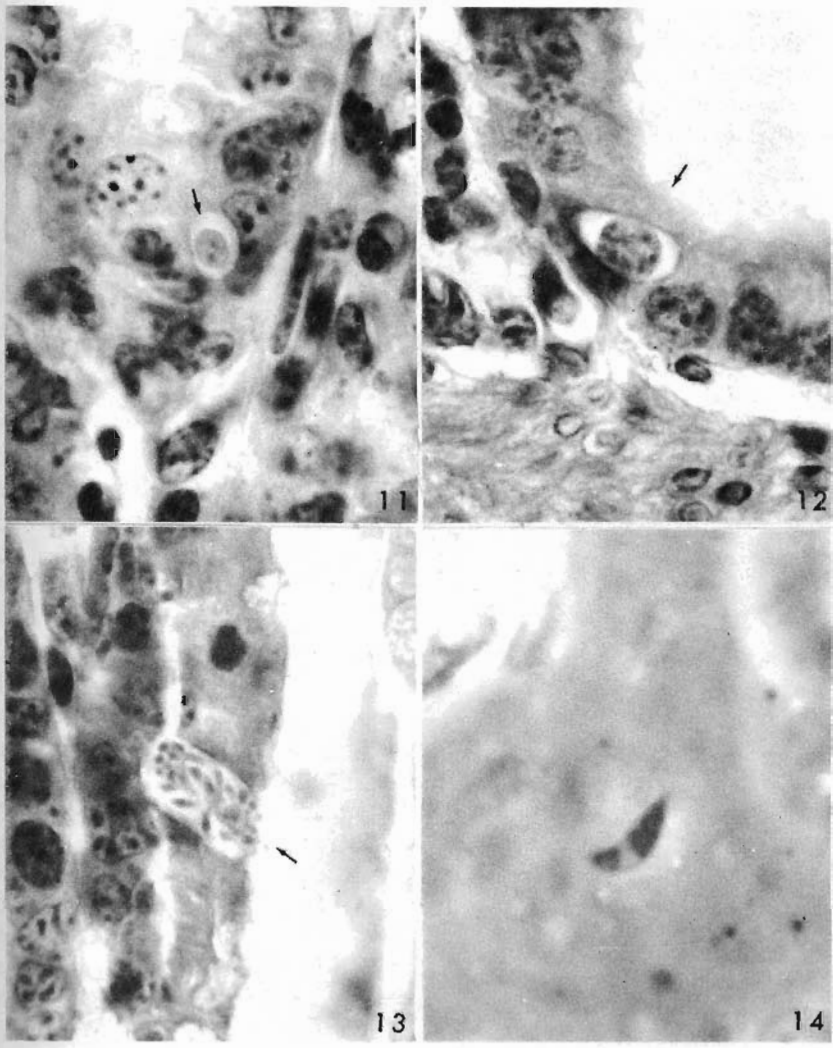

Fig. 11. Early second-generation schizont of *E. bovis;* fixed in Helly's and stained with iron-hematoxylin, X1400. Fig. 12. Intermediate second-generation schizont of *E. bovis;* fixed in Helly's and stained with iron-hematoxylin, X1400. Fig. 13. Mature second-generation schizont of *E. bovis,* containing merozoites; fixed in Helly's and stained with iron-hematoxylin, X1400. Fig. 14. Second-generation merozoite of *E. bovis;* fresh specimen, X2500.

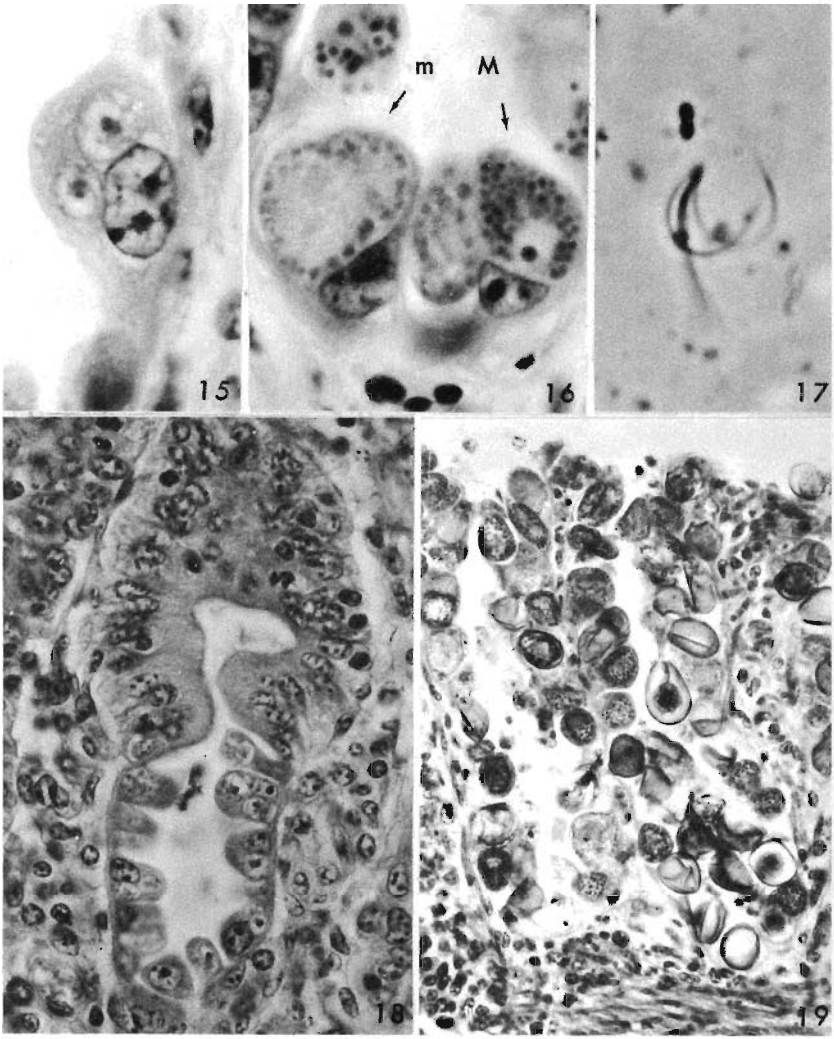

Fig. 15. Two early gametocytes of *E. bovis* in the same host cell; X1400. Fig. 16. Intermediate gametocytes of *E. bovis*, microgametocyte (m) and macrogametocyte (M); X1400. Fig. 17. Microgamete of *E. bovis;* fresh specimen, phase contrast. X3000. Fig. 18. Early gametocytes of *E. bovis*, with alteration of infected cells (below) as compared with normal cells (above); X600. Fig. 19. Oocysts and maturing gametocytes of *E. bovis*, with severe damage to the mucosa; X300. (All figures except 17 from preparations fixed in Helly's and stained with iron-hematoxylin).

This result differed from that of Horton-Smith, Long, and Pierce (1963), who concluded that the merozoites of *E. tenella* penetrate the mucosa of the immune fowl, and that the immune mechanisms are effective only after the merozoites have invaded the host cells. However, our results agree with those of Morehouse (1938), who investigated this question using *E. nieschulzi* in rats.

Another problem in the immunology of bovine coccidia has to do with the extent to which circulating antibodies are involved. Earlier workers were not able to demonstrate such antibodies (Becker, Hall, and Madden, 1935). However, in recent years antibodies have been demonstrated against *E. tenella* in chickens (McDermott and Stauber, 1954; Pierce, Long, and Horton-Smith, 1962), *E. stiedae* in rabbits (Rose, 1963), and *E. meleagrimitis* in turkeys (Augustin and Ridges, 1963). We have been able to show the presence in calves of antibodies against *E. bovis* merozoites and oocysts, beginning about two weeks after exposure to infection (Andersen et al., 1964). These antibodies reached a peak in concentration three to four weeks after exposure, then gradually declined, but were still detectable several months after the last inoculation.

If these antibodies are of importance in the immune reaction it should be possible to passively transfer immunity from an immunized animal to a susceptible one. Thus far, this has not been done successfully in several investigations in cattle (Senger et al., 1959; Fitzgerald, 1964b), rabbits (Rose, 1963), chickens (Pierce, Long, and Horton-Smith, 1963), and turkeys (Augustin and Ridges, 1963). However, in such work negative results do not exclude the possibility that larger amounts of immune blood or serum, or a particular fraction of these, administered at the optimum time with respect to challenge, may have resulted in the transfer of immunity. Rose (1963) has found that globulin from immunized chickens will protect susceptible birds from infection by intravenous inoculation of sporozoites. In such chickens the infective agents would immediately be exposed to the action of the antibodies, whereas parasites inoculated by the usual oral route would not be so exposed. Rose suggested that an increase in permeability of the mucosa to antibody may occur in immunized animals, so that high concentrations of antibody may accumulate at the sites where the parasites invade the tissue. Stauber (1963) has discussed the immunological problems associated with the intracellular location of such parasites as coccidia.

Merozoites of *E. bovis* are adversely affected by serum from immune animals (Andersen et al., 1964). Because they are also so affected, but to a lesser degree, by normal serum from calves, and because degenerative changes also occur in merozoites exposed to saline solutions, it is difficult to determine the degree to which such changes in the merozoites are caused by antibodies. Further work on this problem is needed. Lysis of sporozoites and merozoites of *E. tenella* by immune serum has been reported by Long, Rose, and Pierce (1963), and of the merozoites of *E. meleagrimitis* by Augustin and Ridges (1963).

Problems of interest in the immunology of bovine coccidia which remain to be solved include the nature and role of the antibodies against *E. bovis*. The serum protein fraction containing the antibody or antibodies against *E. bovis* has not yet been determined. The finding of Fitzgerald that increases in alpha globulin accompany severe infections with this species suggests that this component may contain the antibodies. However, antibodies are usually associated with the gamma globulin fraction, and Pierce, Long, and Horton-Smith (1962) found that antibody activity against *E. tenella* in chickens was confined to the serum protein fraction corresponding to gamma globulin in animals. Clark and Smith (1963) concluded from studies with germ-free chickens that the increase in gamma globulin which occurs during cecal coccidiosis is likely caused by microbes other than the coccidia, whereas *E. tenella* did cause an increase in $alpha_2$ globulins. It would also be of value to learn whether antibodies similar or identical to those found in the blood can be demonstrated in the tissues. In attacking this problem, one approach would be to examine the intestinal contents, mucosal washings, mucosa, and intestinal lymph nodes of immune calves for antibodies. If they can be found in these locations, it should be possible to determine whether there is any increase in concentrations of antibody at the site of invasion after exposure to reinfection, resulting from a local sensitivity as suggested by Long, Rose, and Pierce (1963).

Another immunological problem concerns the transfer from infected cells and/or tissues to uninfected cells and/or tissues of the capacity to resist invasion or growth of the coccidia. Becker, Hall, and Madden (1935), in considering the mechanisms of immunity in coccidiosis of rats, inferred from their results that immunity is capable of spreading from centers of infection over the remaining surface of the intestine. No explanation was given as

to how this "spreading" might occur. These authors considered that neither a general systematic response, nor circulating antibodies played a role in this immunity. They presumed that the epithelial cells either acquire the property of blocking the entrance of the sporozoites into their protoplasm, or, by having become sensitized, inhibit the growth of any penetrating sporozoites.

A complicating factor in this problem is the transitory nature of the epithelial cells lining the intestinal wall. These are the host cells for some or all of the stages of the bovine coccidial species whose life cycles are known. In the small intestine there is a continual sloughing off of cells at the tips of the villi. These are replaced by cells which have originated from divisions continually occurring at the base of crypts. Any explanation of the transfer or spread of immunity among these cells must take their impermanent nature into account.

The more recent work showing that a generalized systemic response and circulating antibodies do in fact exist as a result of coccidial infections in chickens, rabbits, turkeys, and cattle has not yet provided a solution to this problem, because there is no proof that these antibodies are actually protective. If systemic, or circulating agents do play a part in the immune reaction, the problem of transfer of immunity to parts of the intestine not affected by the immunizing infection is simplified. It has been suggested on the basis of investigations in chickens and rabbits that the circulating agents active in the immune reaction are cellular elements, such as lymphocytes or plasma cells, rather than antibodies (Horton-Smith et al., 1963). Convincing evidence supporting a cellular basis, possibly associated with the thymus, for the acquired resistance of birds to coccidiosis has recently been reported by Long and Pierce (1963). The cellular aspect of immunity in bovine coccidiosis has not yet been investigated; this is a promising approach for future work.

TRANSMISSION

Coccidia are transmitted from one host animal to another by means of the oocyst stage. So far as is known, only sporulated oocysts are infective. There would appear to be little complexity in explaining the occurrence of sporulation of oocysts after they are discharged in the feces of infected animals, and the initiation of new infections in susceptible animals by ingestion of sporulated oocysts with contaminated food or water. However, there

are good reasons for believing that transmission of coccidial infection does not occur in such a simple, straightforward manner.

One complexity in the transmission of bovine coccidiosis is its occurrence during cold weather. Winter coccidiosis occurs in the western Great Plains and mountainous areas, where severe winter weather commonly prevails. The disease has frequently been observed in the western United States and Canada, and most often affects animals between six months and one year in age.

Because of difficulty in reproducing the disease experimentally, Roderick (1928) suggested that accessory or predisposing factors must be involved in addition to the ingestion of infective oocysts. Elaborating on this suggestion, Marsh (1938) theorized that coccidia are normally present in the intestine of cattle, but do not cause any appreciable damage until the resistance of the host is decreased as a result of exposure to cold, change in feed, or other factors. Thus, no ingestion of oocysts would be required immediately before the outbreak of the disease. However, Boughton (1944) presented arguments against this theory, based upon his observations of coccidiosis in the southeastern United States. He proposed that the occurrence of coccidiosis was explainable on the basis of an accumulation of parasites associated with the overcrowding of susceptible hosts, and pointed out that the known facts concerning coccidial life cycles did not agree with the ideas of Marsh. Marquardt (1962) reported evidence supporting the importance of predisposing factors. He found that Holstein calves which had previously experienced little or no coccidial infection remained nearly free of coccidia for 79 days during the winter, after being placed in the same pen with Hereford calves which were discharging appreciable numbers of oocysts throughout this period. After this time the Holstein calves showed a continuing infection. No explanation could be given as to why the Hereford calves maintained infections while the Holstein calves in the same pen did not. Marquardt considered that his findings provided evidence that winter coccidiosis has its origin in some condition other than exposure of the host to large numbers of infective oocysts. He suggested that sufficient warmth in winter might be provided by the bodies of cattle for sporulation of the oocysts in the fecal material which accumulates on their hair coats. A small percentage of the oocysts found in such situations was found to have undergone sporulation. Smith and Davis (1963) reported the occurrence of an infection in a lamb which probably resulted

from licking sporulated oocysts from the wool of two infected lambs during transportation in a station wagon over a distance of 180 miles.

We obtained results similar in general to those of Marquardt in attempting to obtain transmission of coccidia among calves housed in the same pen during the winter months (Hammond, Sayin, and Miner, 1964). Experimentally infected Holstein calves were housed with uninfected calves for periods of six weeks. No evidence of any transmission of infection was observed. In two experiments, conducted during the winter, sporulation of the oocysts discharged by the infected calves evidently did not occur because of low temperature. However, the uninfected calves in one of the experiments evidently acquired natural infections during a period of six weeks after the conclusion of the experiment, because they did not respond to an inoculation at the end of this period. They may have obtained sporulated oocysts by licking their own bodies or those of their pen-mates, as suggested by Marquardt (1962).

In a study of coccidia in Hereford calves on summer and winter ranges and in feedlots in Utah, Fitzgerald (1962b) found a marked increase in the incidence of infections caused by *E. zurnii* and *E. bovis* during the fall and winter. He was able to recover only a few infective oocysts from the feedlots where outbreaks of coccidiosis were occurring, thus supporting the hypothesis of Marsh. Fitzgerald concluded that aspects of both this hypothesis and that of Boughton may be necessary in explaining the occurrence of winter coccidiosis.

Recently, it has been shown that certain coccidia of chickens and rats could initiate infections if injected intraperitoneally, intravenously, or subcutaneously. In studying the effect of parenteral inoculations of oocysts in cattle, Fitzgerald (1962a) found evidence that intraperitoneal inoculations of *E. bovis* resulted in infections, but in later work (1964c) he determined that such infections were probably the result of accidental inoculation into the intestine.

An important aspect of the problem of transmission of coccidia is the susceptibility of the host to such infection. In calves less than one month old, the response to inoculation with *E. bovis* is relatively uniform. However, in the relatively little work we have done with older animals, these frequently failed to become infected after inoculation, probably as a result of natural infections

which they had acquired. At present we know little about susceptibility to coccidiosis in calves of different age groups and how this is affected by natural or experimental infections. Rabbits become less susceptible to infection with *E. intestinalis* as they grow older (Beyer, 1963), and this holds true also for *E. meleagrimitis* infections in turkeys (Warren, Ball, and Fagg, 1963), but not for coccidia of chickens (Davis, Joyner, and Kendall, 1963).

Fitzgerald[2] has found that daily inoculation of calves over a period of seven weeks with 100, 1000, or 15, 000 oocysts of *E. bovis* and *E. zurnii* resulted in the development of immunity, indicating that relatively few oocysts are required to stimulate resistance to reinfection. Further work is needed with still smaller numbers of oocysts to determine whether a minimum number necessary for development can be established, and whether animals may remain infected indefinitely if given prolonged inoculation of oocysts below this level. Such information would make a significant contribution to our understanding of coccidiosis.

Fitzgerald[2] also found that oocysts inoculated in dry grain were infective, even after storage with the grain for several months. This interesting finding suggests the need for further work on the survival of oocysts as related to the moisture content of their surroundings. It has generally been considered that oocysts are more susceptible to drying than to other kinds of unfavorable environmental conditions. If the ability of oocysts to withstand absence of moisture is confirmed, transmission by licking the hair coat, and even through air currents, seems likely. This finding also suggests the feasibility of immunizing calves by giving small numbers of oocysts in the feed. The use of irradiated oocysts for such a purpose might be worthwhile, as indicated by the success of such methods in controlling lungworms in calves (Poynter, 1963).

TREATMENT

The treatment of bovine coccidiosis is difficult because, as a rule, the signs of the disease do not become noticeable until it is far advanced. In coccidiosis caused by *E. bovis*, the first signs usually occur about seventeen or eighteen days after ingestion of oocysts. At this time the portion of the life cycle within the host has been nearly or entirely completed, with some damage to the

[2]Personal communication, 1964.

intestinal mucosa having already occurred. Thus, treatment administered at this time can at best result in a lessening of the signs of coccidiosis. However, if various drugs are given at an earlier stage of the disease, the clinical signs of infection are largely or entirely prevented.

Boughton (1943) obtained favorable results with sulfaguanidine in experimental infections with *E. bovis* when the drug was given daily for 21 days beginning two days after inoculation, or for eight days beginning 13 days after inoculation. Unfavorable results were obtained when the drug was given for eight days beginning three days before or five days after inoculation. These results indicated that the stages occurring in the late portion of the life cycle are more susceptible to such drug action than those occurring in the early portion. In attempting to determine which part of the later cycle included the most susceptible stages, we found that if certain sulfa drugs were administered 13 to 17 days after inoculation few or no signs of coccidiosis occurred, and few oocysts were discharged (Hammond et al., 1956). However, treatment beginning 18 days after inoculation was ineffective. Thus, it was shown that the stages of *E. bovis* which occur 13 to 17 days after inoculation apparently are the most susceptible to treatment. In later work (Hammond et al., 1959), we observed that a single dose of sulfonamides 13 days after inoculation of *E. bovis* oocysts effectively controlled coccidiosis, as did two smaller doses 12 to 14 days after inoculation. Thus, the period in the life cycle during which the first-generation schizonts are maturing and releasing merozoites appears to be the most vunerable to treatment with sulfa drugs. It was also found that such treatment did not interfere with development of immunity.

Such information still does not solve the problem caused by the usual recognition of the presence of the disease in an animal or herd only after completion of the stage or stages most susceptible to treatment. In further work on this problem we found that nicarbazin and nitrofurazone, which had been found effective in preventing coccidiosis in chickens, were not useful for this purpose in cattle (Hammond et al., 1958; Hammond, Ferguson, and Miner, 1960; Hammond, Sayin, and Miner, 1964). Studies have recently been completed with amprolium, which is one of the newer drugs highly effective against the coccidia of chickens. Amprolium is an antagonist of thiamine, one of the essential vitamins, thus interfering with the metabolism of the parasites. This drug

was found to control coccidiosis caused by *E. bovis* in calves when given in the milk for three weeks beginning on the day before inoculation, or for five days beginning 13 days after inoculation, but not when given in a single dose 13 days after inoculation.[3] The finding that this drug is effective in preventing coccidiosis in cattle indicates that it may be useful for this purpose in special situations in which the disease frequently occurs. Also treatment could be started in all members of a group of animals at the time coccidiosis is first diagnosed in one or more individuals in the group, and could be continued as long as necessary to provide control.

A more precise determination of the stage or stages of *E. bovis* which are most susceptible to treatment is needed. One approach to this would be the use of calves with intestinal cannulas, enabling the periodical removal of samples from the intestine. This has been tried with two calves treated with sulfa drugs on the 13th day after inoculation. In samples of intestinal contents removed 16 days after inoculation from these calves through cannulas placed in the cecum, no merozoites were found in one calf, while in the other calf only inactive, apparently dead merozoites were seen. This preliminary finding indicates that such an approach would yield useful information as to the effect of treatment on the parasites.

Other information which is still needed concerning treatment includes determination of any differences in susceptibility to treatment of *E. bovis* and *E. zurnii*, and the obtaining of information as to whether resistance to certain drugs occurs in bovine coccidia, as has been demonstrated for those of poultry.

SUMMARY

Coccidiosis in cattle is an important disease because it is widespread in occurrence and causes serious losses in mortality and retardation in growth. The most important species of coccidia causing such losses are *Eimeria bovis* and *E. zurnii*. Although much has been learned about coccidial infections in cattle, many important problems remain to be solved. These have to do with the life cycles of these coccidia, excystation, invasion of host cells, effect on the host, immunology, transmission, and treatment.

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THIRTIETH FACULTY HONOR LECTURE DELIVERED AT THE UNIVERSITY

November 19, 1964

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The administration of the University is sympathetic with these aims and shares the cost of publishing and distributing these lectures.

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